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RESEARCH ARTICLE

Investigating the Effects of Controlled Cold Exposure on Brain Cell Integrity and Depression-Related Gene Expression

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ABSTRACT: Cold water therapy, which belongs to hydrotherapy, exposes patients to brief cold water treatments. It enhances immune function and metabolism while reducing pain from inflammatory conditions. This research examined how cold-water exposure affects both brain cell integrity and depression-related gene expression patterns in human subjects. The brain cell cultures were treated with DMEM culture media solution. Then, they were exposed to 4°C temperatures for 5, 15, 30, and 45 minutes under control conditions where untreated cells remained at 37 °C. Then, microscopic imaging was used to examine cell structure and survival rates. Then, the quantitative PCR was performed to measure CREB and BDNF gene expression levels. These results showed that cells maintained their morphology and viability after 45 minutes of cold exposure, yet cell size decreased proportionally to the duration of exposure. The mRNA levels of CREB and BDNF increased substantially (3–4-fold and 2–3-fold, respectively) in cells exposed to 45 minutes of cold treatment, indicating the activation of stress-protective signaling pathways. The research demonstrates that properly managed cold-water exposure can protect brain cells while altering depression-linked molecular markers, which supports its potential use as a depression treatment method.

KEYWORDS: Neuroscience, Cellular and Molecular Neuroscience, Cold Water Therapy, Brain Cell Integrity, Depression-Related Gene Expression.

Introduction

Cold water therapy is a form of hydrotherapy treatment that exposes patients to cold water for a short time.[1] It is widely regarded as physiologically beneficial for the human body as it enhances immune functions and metabolism and alleviates pain from inflammatory conditions such as muscle tension, asthma, and rheumatism.[2] To reduce muscle stiffness, mitigate post-exercise damage, and increase strength gains, athletes frequently treat themselves with cold water immersion therapy and cryotherapy.[3] Accumulated days of cold showers also provide non-athletes with reductions in general sickness.[4] Although cold water therapy provides a more exhaustive exposure to coldness, both cold showers and cold-water therapy allow the human body to experience the advantages of cold shock to the body.[5]

Cold water therapy contains effects beyond just its physical benefits. Many researchers have suggested cold water therapy as a potential treatment for depression, one of the most common yet fatal mental disorders in the world. Although the exact causes for depression are ambiguous, scientists predict that one major cause may be the lack of physiological stressors, such as changes in body temperature. Short-term exposure to coldness creates anti-depressive effects by activating the sympathetic nervous system. This mass release of noradrenaline, combined with the high density of cold receptors, sends many electrical impulses, diminishing the feeling of persistent sadness, emptiness, loneliness, fatigue, and futility.[6] It is also uncovered that cold water therapy encourages the release of hormones and neurotransmitters related to the moderation process of stress, such as Dopamine, serotonin, cortisol, norepinephrine, and β-endorphin. Cold water therapy also increases the interaction between the limbic structures of the brain. The medial and left rostral prefrontal cortices, left anterior insula, and anterior cingulate cortex begin to engage more frequently, resulting in an energetic and less distressed state of mind.[7]

Cell integrity is the unharmed or complete condition of a cell's structure and function. Cell injury occurs when cells are exposed to an overwhelming amount of external or internal stress to the point where they cannot sustain their lives without suffering damage. To a certain extent, cell injury is reversible; however, it eventually transitions to irreparable harm. Consistent or excessive cell injuries may lead to cell death.[8] Countless studies have been conducted that address the effect of cold-water therapy on muscle cell integrity. Yet, few researchers are available to investigate the relationship between cold water therapy and brain cell integrity. Even amongst the small number of studies related to brain cell integrity, researchers disagree with each other. Many studies suggest that cold water therapy is beneficial overall. Treatment such as cold-water swimming rapidly increases endothelial progenitor cells (EPC) and angiogenesis in the blood circulation and hippocampus. This augmented generation of new vessels, usually active during cognitive development, improves recovery of cognitive functions previously damaged from brain trauma.[9] On the contrary, many other studies have discovered that exposure to cold water can alter brain cells, decrease attention span, impair memory, and slow the response and decision-making speed.[7]

Current studies that investigate the effect of cold-water therapy on brain cell integrity and depression face numerous limitations despite the depth of each study. Many studies have

DOI: 10.36838/IJHSR813.54

experimented with non-human entities, typically rodents such as mice. Since human brains are larger, have a more complicated structure of the neocortex, and contain a higher number of specialized regions, the experiment results may not be completely applicable to humans.[10]

The studies are also frequently based on qualitative research done through surveys, observations, and interviews with test subjects. Although this method is beneficial in obtaining a general idea of the benefits and drawbacks of cold-water therapy, quantitative methods are necessary to discover the exact effects on brain cells. There is rarely any practical research done on brain cell integrity. Moreover, the time and temperature utilized by each scientist vary. This factor likely leads to disparities between the results of many experiments, as excessive exposure to stimuli is rarely beneficial for the brain.

This study aimed to investigate the effect of cold-water therapy on human brain cell integrity and depression. We hypothesized that cold temperatures may enhance the human brain cell integrity and inhibit the genes associated with depression.

Methods

Cell culture and maintenance:

A172 human brain glia cells (ATCC) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin to ensure optimal growth and prevent microbial contamination. After a four-day interval, the cells were then incubated at 37˚C with 5% CO_2 for the respective exposure times. In between each experiment, the cell culture media were refreshed every 48 hours to provide the required nutrients.

Cold exposure test condition with microscope imaging:

Before any experiment was done, photos of 8 different cell samples were taken through the microscope at 40X magnification under standardized illumination settings. Then, two conditions were prepared for the cold exposure test. A control condition without cold exposure was maintained at 37˚C, and an experiment group was subjected to cold temperature exposure at 4˚C. The exposure durations were varied: 5, 15, 30, and 45 minutes to analyze the time-dependent effects. The temperature accuracy was ensured by continuous measurement with a calibrated digital thermometer. After the cold treatment, the cells were returned to the standard incubator (37˚C with 5% CO_2) for a recovery period of three days. After the recovery, the same imaging condition was used to image for direct comparison with baseline data.

Cell Viability Analysis subsection:

Cell viability was assessed using the LUNA-FL live cell counter device following cold exposure. Viable cells were quantified according to the manufacturer's protocol, and viability was expressed as the percentage of live cells relative to the 37˚C control group.

Gene expression analysis:

After imaging, total RNA was then extracted from the control and experimental groups using quantitative PCR (qPCR) using the Total RNA extraction kit (Bioneer). RNA yield and purity were quantified using a NanoDrop spectrophotometer. The cDNA synthesis kit (Enzynomics) was used to synthesize cDNA from mRNA. The specific primers targeting CREB and BDNF were designed based on published sequences. GAPDH was used as the internal control for normalization. The qPCR protocol included an initial denaturation step at 95˚C for 10 minutes, followed by 40 cycles of 95˚C for 15 seconds and 60˚C for 10 seconds. All reactions were run in triplicate to ensure reproducibility.

ImageJ analysis of agarose gel-based RT-PCR result:

After the amplified bands were produced using agarose gel electrophoresis, the band intensity was analyzed using the ImageJ program. After the band intensity of each band was quantified, GAPDH intensity was used to normalize the CREB and BDNF mRNA expression levels.

Statistical analysis:

All data are presented as mean ± standard deviation. Statistical comparisons between two groups were performed using an unpaired two-tailed Student's t-test. For comparisons involving more than two groups or multiple time points, a one-way ANOVA followed by Tukey's post-hoc test was used. A p-value of < 0.05 was considered statistically significant. All experiments were performed using three independent biological replicates, and each measurement was obtained from triplicate technical replicates unless otherwise stated.

Results and Discussion

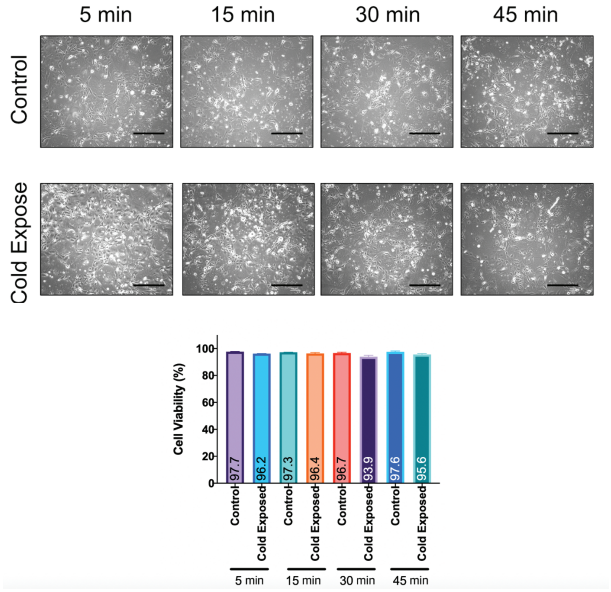

Figure 1: Cold exposure for up to 45 minutes showed no change in cell morphology and viability. The cell image and cell viability of brain cells with the control condition (without cold exposure) and cold exposure (cold exposure at 4 ˚C) for 5, 15, 30, and 45 min (Scale bar = 1000 µm). This result confirms that cold exposure for up to 45 minutes does not impair brain cell survival or basic structural integrity, establishing it as a safe window for further molecular investigation.

Since exposure time is one of the significant variables affecting brain cell conditions, the optimal cold exposure time must be tested. This experiment aimed to discover the optimal time for cold water exposure that has no effect on cell viability but has a positive effect on maintaining brain integrity. The brain cells were induced at 5, 15, 30, and 45 minutes each. Cell images were captured using a microscope before and after the cold exposure to measure the cell viability. Both cold-exposure cells and control cells were measured after each time interval to compare the overall cell integrity. Based on the cell images taken through the microscope, no significant morphological change was observed in the cells after the experiment (Figure 1). The cell viability remained relatively similar throughout the experiment as well. The control group's cell viability was approximately 97.3%, with data collection of 97.7%, 97.3%, 96.7%, and 97.6% chronologically. The lowest was 93.9%, and the highest was 96.2%, and there were no significant fluctuations in the cell viability for the four cold exposure sample groups either. The sample that was exposed to the cold for the longest time, 45 minutes, showed only a 0.1% difference in average cell viability (95.5%). Barely any change was observed in the cell morphology and viability between the cold exposure group and the control group at four time intervals. Thus, it is concluded that a 45-minute cold exposure time is optimal for further experiments as it maximizes the time the cell is exposed to the cold while keeping the cell integrity unchanged. Cold exposure therapy has no effect on brain cell morphology and viability for durations up to 45 minutes.

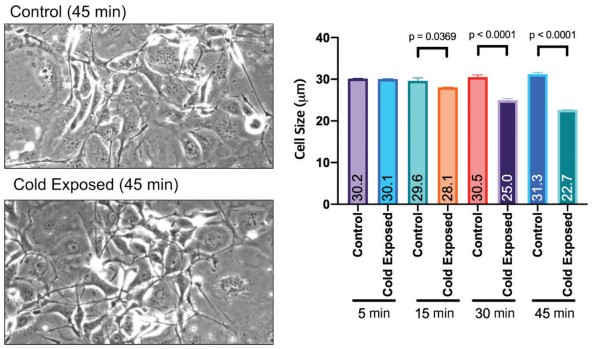

Figure 2: Cold exposure for 45 minutes showed a visible change in cell integrity. The cell image and size comparison graph of brain cells with the control condition (without cold exposure) and cold exposure (cold exposure at 4 ˚C) (Scale bar = 1000 μm). These results demonstrate a significant, time-dependent reduction in glial cell size following 4°C exposure. While the control group maintained a mean size of μm, the 45-minute treatment group decreased to μm ($p < 0.0001$), suggesting structural adaptation to thermal stress without a loss in overall cell viability. All quantitative data are now reported as mean ± standard deviation together with corresponding p-values. At 5 minutes, no meaningful difference was observed between the control and cold-exposed groups (30.2 ± 0.2 μm vs. 30.1 ± 0.2 μm). At 15 minutes, the cold-exposed group showed a modest but significant decrease in mean cell size compared with the control group (28.1 ± 0.3 μm vs. 29.6 ± 0.6 μm, $p = 0.0369$). This reduction became more pronounced at 30 minutes (25.0 ± 0.4 μm vs. 30.5 ± 0.7 μm, $p < 0.0001$) and 45 minutes (22.7 ± 0.2 μm vs. 31.3 ± 0.3 μm, $p < 0.0001$).

Since no changes were observed in the cell morphology and viability in the cell under 45 minutes of cold exposure time, this experiment aimed to evaluate the proper effects of cold exposure on the cell integrity, specifically the cell size. As the exposure time increases, there is a significant visible and statistical change in the size of each brain cell (Figure 2). The general cell size of the control group is about 30.4 μm, with data of 30.2 μm, 29.6 μm, 30.5 μm, and 31.3 μm. At 5 minutes, the control and experimental samples have only a 0.1 μm difference. After 15 minutes, the experimental sample's cell size decreases by 2 μm from the size at 5-minute exposure ($p=0.0369$). At 30 minutes, the sample decreases again by 3.1μm ($p<0.0001$), and finally at 45 minutes, the sample size decreases by 2.3 μm from the previous data, leaving the final cell size of the cold exposure cell as 22.7 μm ($p<0.0001$). In comparison, the final cell size of the control cell is 31.3 μm. The experiment data show that exposure to the cold decreases cell size, impacting cell integrity. The observed pattern of cell size decreasing as cold exposure time increases establishes the credibility of the experimental data. This experiment concludes that 45 minutes is the optimal time for cold exposure, as no changes are observed in cell morphology and viability. Yet, it makes the most impact on cell integrity.

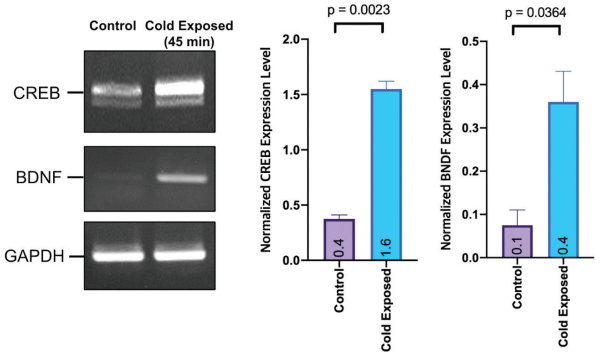

Figure 3: Cold exposure increases CREB and BDNF expression in brain cells. (Left) Representative RT-PCR gel images showing CREB, BDNF, and GAPDH expression levels in control and cold-exposed (45 min) brain cells. (Right) Quantification of CREB and BDNF expression levels normalized to GAPDH. RT-qPCR analysis reveals that 45 minutes of exposure to 4°C significantly upregulates depression-linked markers, specifically increasing CREB expression by 3–4-fold ($p = 0.0023$) and BDNF expression by 2–3-fold ($p = 0.0364$) compared to 37°C controls.

The RT-qPCR analysis showed a significant upregulation of CREB and BDNF mRNA levels after cold exposure in brain cells (Figure 3). Simultaneously, the CREB expression increased approximately 3–4-fold ($p = 0.0023$), while BDNF expression increased 2–3-fold ($p = 0.0364$) compared to controls. Normalization to GAPDH confirmed the reliability of these findings. These results ultimately indicate that cold exposure activates the CREB/BDNF signaling pathway in brain cells.

CREB and BDNF are crucial in depression research as BDNF supports neuroplasticity, neuronal survival, and synaptic remodeling, while CREB is a transcription factor that regulates BDNF expression and is involved in antidepressant mechanisms. Thus, depression is associated with low BDNF and impaired CREB signaling in brain regions like the hippocampus. Cold exposure may exert antidepressant effects by enhancing neuroplasticity, which is visible through CREB activity. The increase in CREB activity likely promotes BDNF transcription, improving neuronal function. Cold exposure

DOI: 10.36838/IJHSR813.54

may also be a mild stressor that triggers adaptive neuroprotective mechanisms (hormesis). Similar mechanisms have been observed with antidepressant treatments, which also upregulate CREB and BDNF.

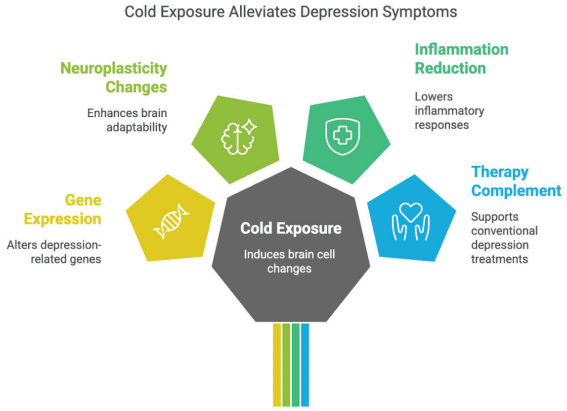

Figure 4: Summary illustration of the mechanisms by which cold exposure may alleviate depression symptoms. Cold exposure induces brain cell changes that contribute to several neurobiological benefits relevant to mental health. These include: (1) Neuroplasticity Changes, enhancing brain adaptability and resilience to stress; (2) Gene Expression, modulating genes associated with depression; (3) Inflammation Reduction, decreasing systemic and neural inflammatory responses often linked to depressive symptoms; and (4) Therapy Complement, serving as an adjunct to conventional depression treatments by reinforcing their therapeutic effects. This visual encapsulates the multifaceted benefits of cold therapy and links molecular and cellular responses to broader psychological improvements.

Despite the promising findings of this research, its limitations must be acknowledged. First, while A172 human glial cells present a close representation of human behavior, they are not an exact reflection of the actual brain. The intricate structure of the brain, including the cellular networks in each region, allows for potential errors. Secondly, this study uses a restricted methodology, such as a limited duration (45 minutes), a single temperature as the cold exposure setup, and only investigating short-term effects. Whether different thermal conditions would produce varying results in this experiment is unknown. Thirdly, as depression is a polygenic disorder, only utilizing BDNF and CREB genes as key markers leaves the potential effects of cold exposure underexplored.

Going forward, future studies should address these gaps by replicating these findings in *in vivo* models using rodents, then humans, to validate whether the observed molecular changes translate to behavioral antidepressant effects. Broader temperature and exposure duration ranges must be tested, as well as replication of the experiment over time to examine the long-term effects of repeated cold exposure. Moreover, investigating the effects on additional depression-associated markers, such as serotonin transporter expression, Solute Carrier Family 6 Member (SLC6A4), Tryptophan hydroxylase 2 (TPH2), and FK506 Binding Protein 5 (FKBP5), will be necessary to fully assess the extent of cold water exposure's benefits.

Conclusion

This study indicates that controlled cold temperature exposure does not adversely affect A172 human brain cell morphology or viability even after 45 minutes of cold temperature treatment. Instead, it induces a measurable reduction in cell size. Also, a significant upregulation of CREB and BDNF gene expression was observed in cells exposed to cold temperatures. This suggests the activation of neuroprotective and neuroplasticity-related pathways. These molecular changes show that cold water therapy could trigger adaptive stress responses that may control depressive symptoms by enhancing neuronal integrity and functions (Figure 4).

These findings provide a promising mechanistic insight into the potential role of cold-water therapy as a treatment for depression. However, these controlled *in vitro* conditions limit application to human subjects. Therefore, future research should focus on replicating these findings in mouse experiments. Also, the long-term effects of cold exposure and elucidating the underlying molecular pathways need to be analyzed further.

Acknowledgments

I want to thank Prof. Woo Rin Lee from the University of Suwon for his guidance and support for this research paper.

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DOI: 10.36838/IJHSR813.54